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Review

Potential influence of *Nagella sativa* (Black cumin) in reinforcing immune system: A hope to decelerate the COVID-19 pandemic

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ABSTRACT

The world is witnessing a difficult time. The race of developing a new coronavirus (COVID-19) vaccine is becoming more urgent. Many preliminary studies on the pathophysiology of COVID-19 patients have provided some clues to treat this pandemic. However, no suitable treatment has found yet. Various symptoms of patients infected with COVID-19 indicated the importance of immune regulation in the human body. Severe cases admitted to the intensive care unit showed high level of pro-inflammatory cytokines which enhanced the disease severity. Acute Respiratory Distress Syndrome (ARDS) in COVID-19 patients is another critical factor of disease severity and mortality. So, Immune modulation is the only way of regulating immune system. *Nigella sativa* has been used for medicinal purposes for centuries. The components of this plant are known for its intense immune-regulatory, anti-inflammatory, and antioxidant benefits in obstructive respiratory disorders. A molecular docking study also gave evidences that *N. sativa* decelerates COVID-19 and might give the same or better results than the FDA approved drugs. The aim of this review was to investigate the possible immune-regulatory effects of *N. sativa* on COVID-19 pandemic. Our review found *N. sativa's* Thymoquinone, Nigellidine, and α-hederin can be a potential influencer in reinforcing the immune response on molecular grounds.

Introduction

In previous some decades, several life-threatening viruses have originated. These viruses caused significant deaths with serious health concerns around the world. Outbreaks of these viruses could happen anywhere due to the vast movement of people and goods transport (Al-Hazmi, 2016). Coronavirus is one of the widely distributed family of viruses among humans, and animals (Li et al., 2006). These type of viruses cause respiratory, enteric, hepatic, and neurologic diseases in human with high prevalence (Zhu et al., 2020). These may transmit through respiratory droplets and cause common cold (7–30%) with a peak prevalence in late fall, winter, and early spring (van der Hoek et al., 2004). After the onset of Severe Acute Respiratory Syndrome (SARS) in 2003, coronaviruses were identified as "emerging pathogen" (Weiss and Navas-Martin, 2005) with high level of genetic diversity, frequent genomic rearrangements and widely distribution between human and animals (Cui et al., 2019; Wong et al., 2015). On June 13, 2012, a new strain of coronavirus (MERS-CoV) was again reported in the Saudi Arabia especially in Makkah, Jeddah, Riyadh, and Al-Hassa cities. This new strain infected a big number of people and caused many deaths in Saudi Arabia (Al Mutair and Ambani, 2019). The virus spread to other nearby countries in middle east, including Qatar, Kuwait, Jordan, Bahrain, and Tunisia. Due to the Infected travellers, virus also spread to Southeast Asia, North Africa, Europe, and United States (Timen et al., 2012). In September 2018, World Health Organization (WHO) reported MERS-CoV outbreak in approximately 27 countries around the globe,

Abbreviations: FDA, food and drug administration; IFN, interferon; MAPK, Mitogen Activated Protein Kinase; PKα, protein kinase alpha; PKB, Protein Kinase Beta; ROS, reactive oxygen species; TBK1, TANK-Binding Kinase 1; TQ, thymoquinone.

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with 2279 confirmed cases and 806 deaths (Mubarak et al., 2019).

Now again world is under the threat of 2019 novel coronavirus. This outbreak poses a serious threat to health care. Authorities has prompted to step up surveillance against the emergence of this virus (Hui et al., 2020). Authorities took very reasonable and timely measures, such as active case detection, follow-up investigation, sanitation and disinfection of suspected areas. Public awareness campaigns were also hold to raise self-protection measures (Weiss and Navas-Martin, 2005). But besides all these measures, virus is still spreading throughout the world, till now infections have been identified in 195 countries and caused thousands of deaths. On the basis of its spread and severity, on March 11, 2020 WHO characterised the COVID-19 situation as a pandemic and ask the countries to do best against the spread of virus (Bedford et al., 2020). But so far, apart from supportive therapy, no specific treatment against this virus has proven effective. Therefore, specific drugs for this disease still have been being studied. Researchers are studying a large number of herbal plants and their components for discovering such specific drugs. The identification of the medicinal components of these plants and their effectiveness in the treatment of diseases can pave the way for major new discoveries in the treatment of COVID-19. Several plants have active biomolecules with proven therapeutic properties. Such plants have been used since ancient times to treat various human diseases (Majeed et al., 2020). *N. sativa* is one of them, it is usually called as black cumin. It has long been used as a condiment and mitigating agent in various diseases (Dubey et al., 2016). *N. sativa* plant has a rich historical and religious background as a magical condimental herb (Ijaz et al., 2017; Ikhsan et al., 2018; Shrestha et al., 2012; Zaidi et al., 2015). This plant is usually grown in the Middle East, Europe, and Asia. It contains small black seeds, known as black cumin or black seed. Thymquinone (TQ) is the most pharmacologically active ingredient in these seeds. Recent pharmacological studies suggested its probable role in many disorders especially in respiratory distress conditions (Hossein et al., 2008; Mohebbati and Abbasnezhad, 2020; Randhawa, 2008; Zaher et al., 2008). As, inflammation of lungs is the main pathophysiological feature of COVID-19 patients, in which immune and oxidative processes are involved. So, by inhibition or modulation of these processes may help to overcome COVID-19 (Danzi et al., 2020; Rothan and Byrareddy, 2020; Zhe Xu et al., 2020). *N. sativa* has the ability to modulate or inhibit such processes (Shaterzadeh-Yazdi et al., 2018). Current review summarised such reinforcing potential of *N. sativa*.

Phytoconstituents of *N. sativa*

The first report on the chemical composition of *N. sativa* seeds was published in 1880 (El-Tahir and Bakeet, 2006). To date, many active compounds have been isolated and identified from this plant species. The general ingredients of this plant are carbohydrates, proteins (Gholamnezhad et al., 2015) and other biochemical components e.g. Thymoquinone (Fig. 1,2).

There are some other trace elements, including vitamins (Niacin, Thiamine, Riboflavin, Folic acid, Pyridoxine, Vitamin E), and minerals (Magnesium, Potassium, Phosphorus, Sodium, Copper, Calcium, and Iron (Ahmad et al., 2013; Amin and Hosseinzadeh, 2016; El-Tahir and Bakeet, 2006; Tembhurne et al., 2014). Most of the pharmacological companies are working on TQ (Thymoquinone) (Forouzanfar et al., 2014). It is widely acknowledged for the broad spectrum medicinal properties including anti-proliferative, gene regulating, anti-oxidant, and protective effect against different viral or bacterial respiratory issues (Majeed et al., 2020).

Generally, the constituents found in black cumin can be divided in two categories; volatile and non-volatile compounds (Oskouei et al., 2018). The volatile contains saturated fatty acids. This portion of seed also contains: t-anethole, p-cymene, 4-terpineol, carvacrol, and longifoline (Ahmad et al., 2013; Enomoto et al., 2001). The other category found in *N. sativa* is non-volatile compounds e.g. alkaloids. The seeds have two different kinds of alkaloids; isoquinoline alkaloids such as nigellicimine and pyrazol that includes nigellidine and nigellicine (Table 1). Moreover, proteins, saponins, fatty acids, carbohydrates, and phenolic compounds such as flavonoids have also been reported (Tavakkoli et al., 2017).

Experimental evidences of *N. sativa* and its constituents in respiratory diseases

A lot of studies showed a preventive and therapeutic effect of *N. sativa* for various respiratory diseases. The clinical effects of *N. sativa* and its constituents on various respiratory diseases are summarized in Table 2.

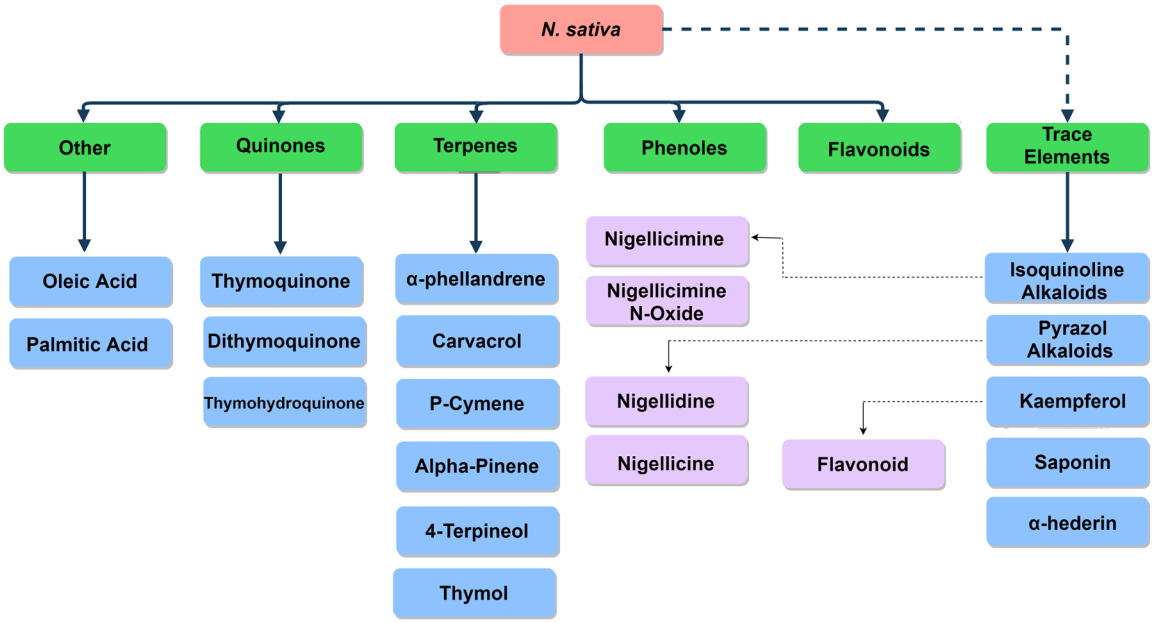

Fig. 1. Biochemical constituents of N. sativa (Ahmad et al., 2013; El-Tahir and Bakeet, 2006; Kooti et al., 2016; Majeed et al., 2020; Tembhurne et al., 2014).

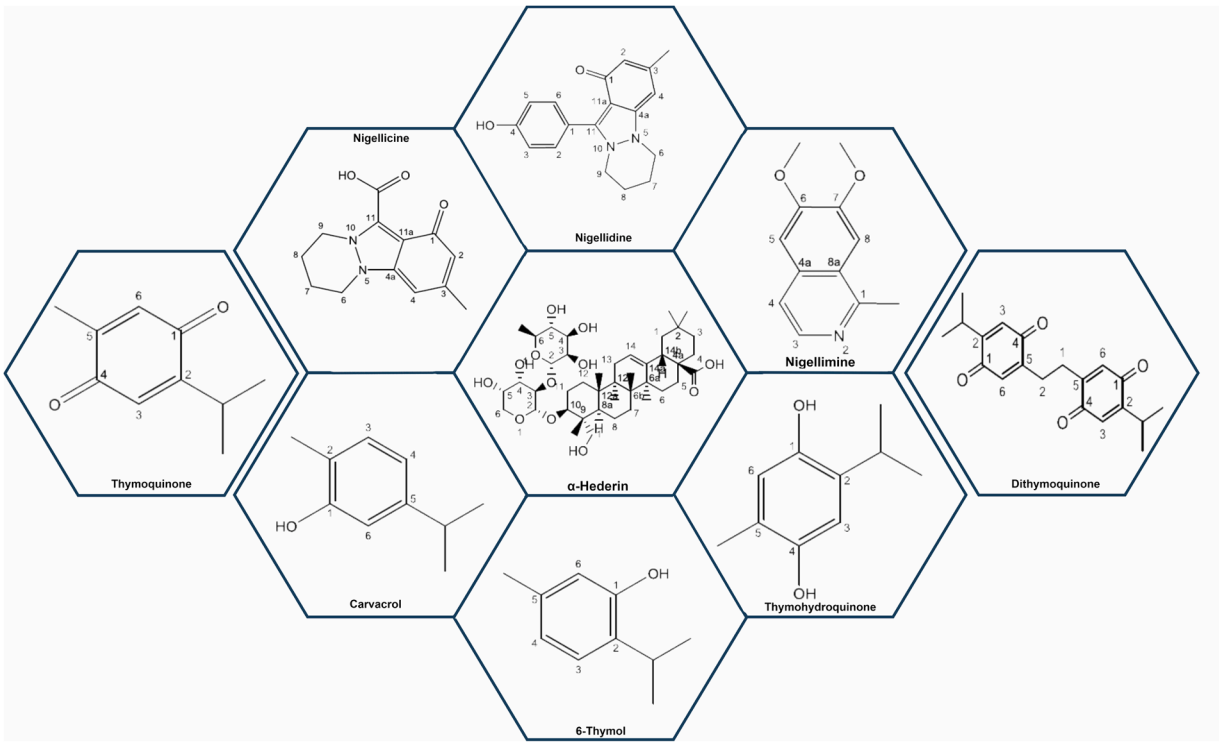

Fig. 2. Chemical structures of major compounds from *N. sativa* (Ghahramanloo et al., 2017; Khan and Afzal, 2016; Kokoska et al., 2008; Muhialdin et al., 2016).

Table 1
The general composition of N. sativa seeds (Khan and Afzal, 2016; Vaz et al., 2018; Venkatachallam et al., 2010).

Constituents	General composition	% Range
Oil	**Fixed Oil:** Omega-6, Omega-3, Oleic acid, Palmitoleic acid, Myristoleic acid, Eicosadienoic acid, Myristic acid, Diho-molionolenic acid, Stearic acid, Sterols (lanosterol, Campesterol, β-sitosterol, Avenasterol, and stigmasterol), Arachidic acid, Behanic acid, Tocopherols (α, β, γ) Retinol, Thymoquinone, and Carotenoids	22–38
	Volatile Oil: Thymoquinone, α-Pinene, *p*-Cymene, Dithymoquinone (Nigellone), Carvacol, Longifolene, β-Pinene, Thymo-hydroquinone, t-Anethole and Thymol	0.40–1.50
Protein	Leucine, Glutamic acid, Lysine, Arginine, Valine, Aspartic acid, Glycine, Histidine, Phenylalanine Iso-leucine, Methionine, and Threonine	20.8–31.2
Carbohydrate	Xylose, Arabinose, Rhamnose, and Glucose	24.9–40
Minerals	Sodium, Potassium, Phosphorus, Calcium, Manganese, Zinc, Selenium, Magnesium, Iron, and Copper	3.7–7
Saponins	α-Hederin, and Hederagenin	0.013
Alkaloids	Nigellidine Nigelicine, and Nigellimine	0.01
Other Vitamins	Riboflavin, Vitamin A&C, Thiamin, Niacin, Folacin, and Pyridoxine	1–4

Preventive effects of *N. sativa* in COVID-19 patients

Inflammation of lungs is the main pathophysiological feature of COVID-19 patients in which immune and oxidative processes are involved (Danzi et al., 2020). So, finding a protective and multi potential drug to stop such respiratory distress is the main goal for effective treatment of COVID-19 (Li et al., 2020). Black seed on the immune system has been investigated by many researchers in respiratory distress conditions. All studies have shown that the *N. sativa* inhibited cyclo-oxygenase (Goyal et al., 2017) and 5-lipooxygenase pathways of acid arachidonic metabolism. Such effectiveness on lung inflammation showed the ameliorating effect of *N. sativa* against leukocytes and eosinophils which can be related to anti-inflammatory and antioxidant properties (Hossein et al., 2008). This medicinal potential is due to nigellone and carbonyl polymer of Thymoquinone (TQ) that inhibits histamine release from peritoneal mast cells by decreasing intracellular calcium through inhibiting protein kinase C and oxidative energy (Gali-Muhtasib et al., 2006).

Anti-inflammatory and immunomodulatory aspects of Thymoquinone

According to the recent reports, a variety of COVID-19 cases admitted to the intensive care unit showed high levels of pro-inflammatory cytokines including TNFα (Tumor necrosis factor alpha), IL-6 and lymphopenia with acute respiratory distress syndrome. As, IL-6 levels significantly correlated with the severity of COVID-19. So, inhibitory effect on IL-6 might be proven as an effective treatment (Liu et al., 2020; Rothan and Byrareddy, 2020). Such inflammatory changes can be easily sorted out through Thymoquinone (TQ) as it has the ability to modulate or inhibit inflammatory responses e.g. IL-1, IL-6, IL-10, IL-18, TNF-α, and NF-κB (Fig. 3) (Shaterzadeh-Yazdi et al., 2018; Srinivasan, 2018)

Thymoquinone (2-Isopropyl-5-methyl-1, 4-benzoquinone) is the main bioactive component of *N. sativa.* It has been found to exhibit numerous activities as anti-oxidant, antihistaminic, antitumor, analgesic, anti-alzheimer, hepatoprotective, neuroprotective, renoprotective, histone protein modulator, insecticidal, and anti-ischemic (Ahmad et al., 2019; Khader and Eckl, 2014).

According to some reports TQ inhibits the 5-lipoxygenase, leukotriene B4, C4, and Th2 cytokines in Broncho alveolar lavage (BAL) fluid with a significantly increase number of eosinophilia and goblet cells in lung tissue (El Gazzar et al., 2006). It also inhibits the expression of inducible nitric oxide synthase's mRNA and transforming growth factor β1 (TGF-β1) (Ammar et al., 2011). Such anti-inflammatory outcome of TQ is caused by increase expression level of hem oxygenase 1 (HO-1) in human keratinocyte (HaCaT), which is activated by nuclear factor (NF) via ROS mediated

Table 2
The experimental evidence of *N. sativa* and its constituents on different respiratory diseases.

Preparation	Dose	Study model	Effect	Reference
Aqueous extract	18.7 mg/kg	Chemical war victims	Improved PFT and respiratory symptoms	(Boskabady et al., 2008)
Alcoholic extract & oil	0.01 to 1 mg/ml	Human lung cancer	Reduced cell viability	(Al-Sheddi et al., 2014)
α-hederin TQ	6–40 μM, 25–150 μM	HEp-2 cellular model	Constrained cell proliferation, evoked apoptosis & necrosis	(ROONEY and Ryan, 2005)
Thymoquinone	5microM	HEp-2 cellular model	Constrained cell numbers	(Womack et al., 2006)
Hydro-ethanolic extract	0.1 g/kg	Cigarette smoke exposed guinea pigs	Protective effect against TR	(Keyhanmanesh et al., 2014)
N. sativa oil	1 ml/kg	Pulmonary fibrosis	Constrained inflammatory index & fibrosis score, Preventive effect against fibrosis	(Abidi et al., 2017)
Thymoquinone	20 and 40 mg/kg	Pulmonary fibrosis	Subdued oxidative stress, Down regulation of pro-fibrotic genes, Preventive effect against fibrosis	(Pourgholamhossein et al., 2016)
Thymoquinone	5 mg/kg	Pulmonary fibrosis	Inhibited NF-Kb, Preventive effect against fibrosis	(El-Khouly et al., 2012)
Thymoquinone	8, 12, 16 mg/kg	Pulmonary artery hypertension	Constrained pulmonary arterial remodelling. Improved hypertension	(Zhu et al., 2016)
N. sativa oil	1.808 μg/kg	Patients have nasal dryness	Improved dryness, Obstruction & crusting	(Oysu et al., 2014)
Hydro-ethanolic extract	50, 100, 200 mg/kg	Rhino-sinusitis	Decreased NO level, Prevented histopathological changes	(Yoruk et al., 2017)
Ethanolic extract	125, 250, 500 mg/kg	CLP induced sepsis	Reduced pro-inflammatory cytokines Reduced oxidative stress markers Reduced histopathology changes	(Bayir et al., 2012)
Aqueous extract	15 mg/kg	Asthmatic patients	Improved asthmatic symptoms, chest wheeze, and PFT values. Reduced the required dosage of inhaler, β-agonists, corticosteroid, and theophylline	(Boskabady et al., 2007)
Seed powder	1 and 2 g (13 mg & 26 mg/kg	Asthmatic patients	Enhanced PFT and ACT score, Increased FEF25–75% & FEV1%. Decreased FeNO & IgE, Increased IFN-γ	(Salem et al., 2017)
Aqueous extract	100 mg/kg	Asthmatic patients	Improved overall clinical symptoms, Elevated FEV1% & FVC/l	(Al-Jawad et al., 2012)
α-hederin	0.02 mg/kg	OVA sensitized rats	Decreased IL-2 & IL-17 mRNA levels. Increased miRNA-133a gene expression.	(Ebrahimi et al., 2016)
α-hederin	0.3 and 3 mg/kg	OVA- sensitized guinea pigs.	Decreased tracheal responsiveness, WBCs & eosinophils.	(Saadat et al., 2015)

PFT; Pulmonary Function Test, ACT; Asthma Control Test, NO; Nitric Oxide, TR; Tar, CLP; Cecal Ligation and Puncture, OVA; ovalbumin, FEV; Forced Expiratory Volume, FEF; Forced Expiratory Flow, FeNO; Fractional Exhaled Nitric Oxide.

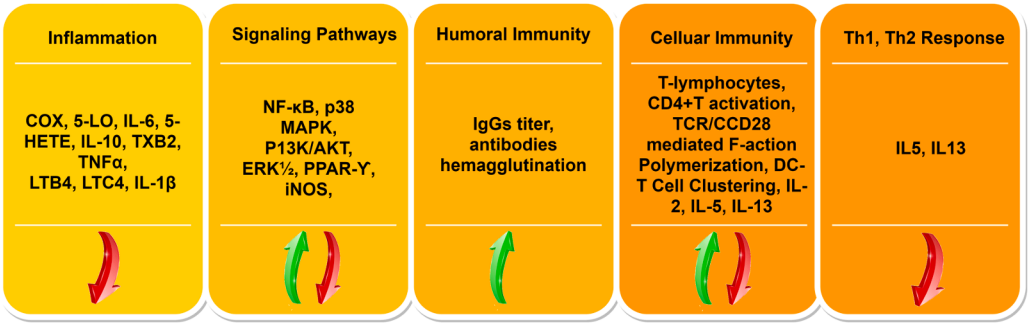

Fig. 3. Thymoquinone responses under the influence of its Immunomodulatory and anti-inflammatory activities (Islam et al., 2019; Majdalawieh and Fayyad, 2015).

phosphorylation of protein kinase B (PKB) and protein kinase alpha (PKα) (Khader and Eckl, 2014). So, it indirectly antagonizes the side effects caused by an increase in ROS level (Mansour et al., 2002). This antioxidant potential of TQ might associated with redox properties of quinone structure and unrestricted competence of TQ to cross substantial barriers to cellular alcove (Darakhshan et al., 2015).

Suppression of IRF-3 mediated expression through Thymoquinone

Interferon 3 regulator (IRF-3) is the most studied member in the family of interferon regulatory transcription factor. It is widely known member in the activation of mitogen-activated protein kinase (MAPK), which plays an important role in activating interferon genes to endogenous viral immunity (Dragan et al., 2007; Hossen et al., 2017). TQ decreases mRNA expression of these interferon genes (IFN-α, IFN-β) through suppression of IRF-3 under the phenomenon of auto phosphorylation of TANK-binding kinase 1 (TBK1). Such a novel insight of anti-inflammatory activity decreases the production of IFN (Fig. 4) (Aziz et al., 2018).

Effect of "Nigellidine" and "α-hederin" as potential inhibitor against COVID-19

Several studies have suggested the medicines that potentially effective for the treatment of COVID-19. These medicines are mostly based on *in vitro* studies, and virtual screenings (Rismanbaf, 2020). As COVID-19 shares sequence similarity to SARS (Ton et al., 2020; Zhijian Xu et al., 2020; Zheng, 2020). So, chemists are also focusing on the main protease of SARS-Coronavirus to develop antiviral treatments (Tang et al., 2020).

N. sativa seeds have wide therapeutic effects against many ailments. These ailments present voluminous confirmation for the biological and biomedical activates. COVID-19 virus has three important proteins, known as papain-like protease (PL^{Pro}), 3C-like protease ($3PL^{Pro}$), and spike protein (SP) as SARS virus. These are the enticing targets for drug development (Zhang et al., 2020). These proteins might be target of *N. sativa's* compounds for identification of favourable molecules in COVID-19 treatment. A molecular docking study showed that "Nigellidine" and "α- hederin" of *N. sativa* inhibited COVID-19 and SARS virus and gave the same or better results than the drugs used in intensive care units to treat patients (Fig. 5,6) (Bouchentouf and Missoum, 2020).

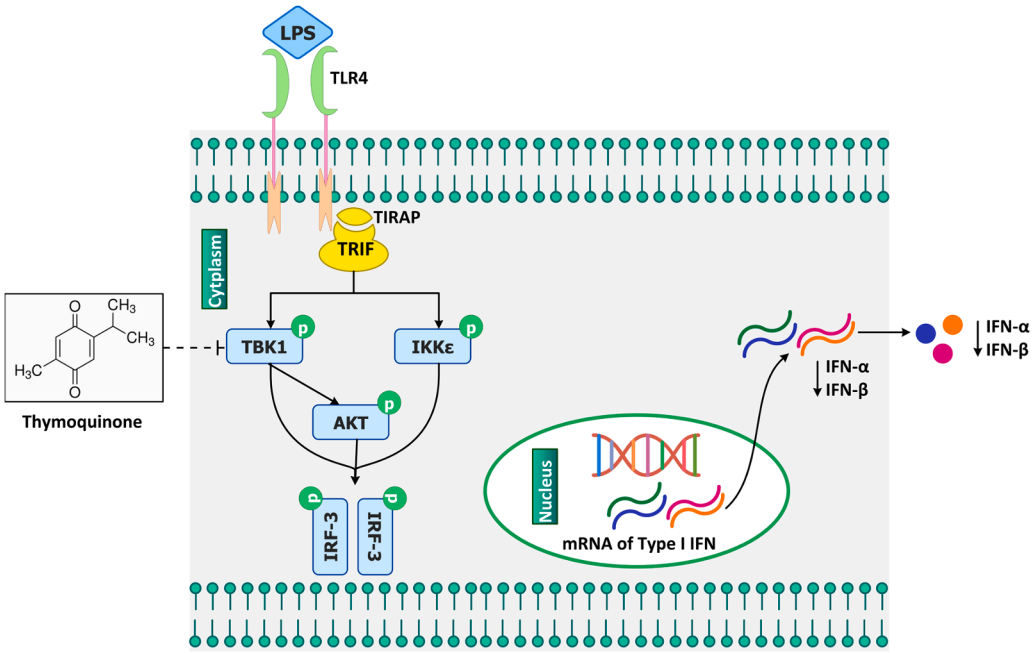

Fig. 4. Suppression of IRF-3 mediated expression through Thymoquinone via downregulation of TBK1.

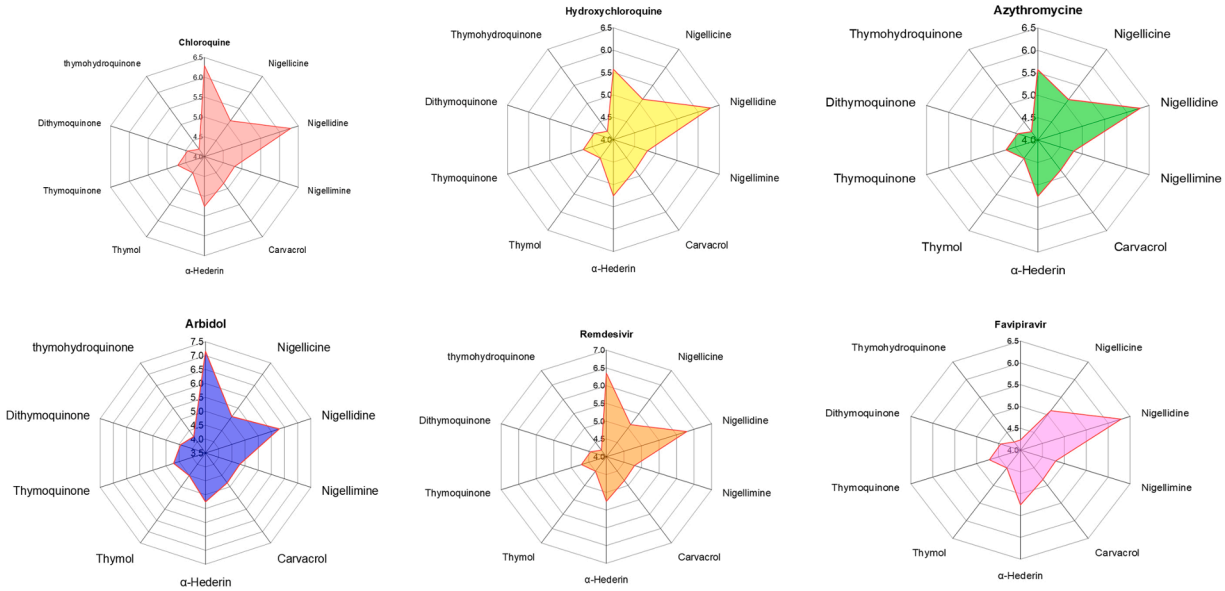

Fig. 5. Comparison between the lowest (negative) energy score obtained from docking of *N. sativa* compounds and FDA approved drugs for the treatment of COVID-19 (Bouchentouf and Missoum, 2020).

Regulation of IL-13 through α-Hederin via miRNA-126 suppression

Acute Lung Injury (ALI) caused by coronavirus infections is attributable to a complex pathophysiological process in which inflammatory cytokines (released by activated alveolar macrophages) induced immune system dysregulation (Gu and Korteweg, 2007; Rothan and Byrareddy, 2020). Such dysregulation of inflammatory cytokines always leads to respiratory failure due to poor oxygenation (Akhtar et al., 2019). MicroRNAs (miRNAs) are much more important in regulating these inflammatory cytokines by negative regulation of different genes expression (Angulo et al., 2012). Many studies demonstrated that miRNAs can modulate essential physiological processes that lead to expression or suppression of a molecular cascade (Fallahi et al., 2016). In viral infections, host antiviral miRNAs play a crucial role in the regulation of immune response to virus infection, depending upon the viral agent (Sardar et al., 2020). MiRNA-126 is amongst the one, being more studied according to the lung inflammation. Because its regulation is directly related to IL-13 expression or suppression (Collison et al., 2011).

Alpha-hederin results in the decline of miRNA-126 expression in lungs due to the raise in β2-adrenergic responsiveness which consequently interfere with IL-13 secretion pathway. It leads to a reduction in inflammatory responses (Fallahi et al., 2016) such as; airway inflammation, airway hyper responsiveness, mucus metaplasia, subepithelial fibrosis and goblet cell hyperplasia (Greene and Gaughan, 2013; Grünig et al., 2012). The α-hederin is also a content in *Kalopanax septemlobus*, which is a traditional and local Chinese medicine use as anti-inflammatory and analgesic in different disease conditions (Bai et al., 2011; Da et al., 2003).

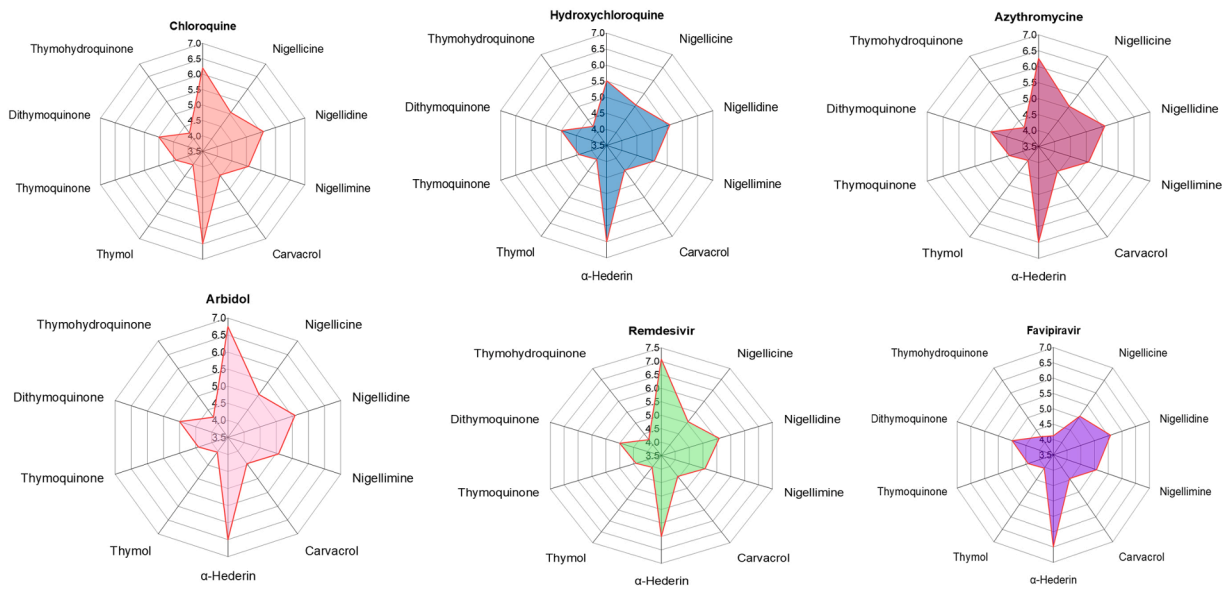

Fig. 6. Comparison between the lowest (negative) energy score obtained from docking of *N. sativa* compounds and FDA approved drugs for the treatment of SARS. (Bouchentouf and Missoum, 2020).

Challenges

Formal drug development for N. sativa

N. sativa clearly defines a unified drug development in order to standardize the pharmaceutical preparations for powdered seeds, extracts, oil, or some of the other active component of seeds (such as thymoquinone) to insure the stability of components. So, it is necessary to determine the pharmacokinetic properties of such components (Dajani et al., 2018). In addition, the maximum permissible dose for humans and the safety of certain medical applications should also be considered. However, development priorities for a specific application require close collaboration among clinical researchers, pharmacists, and governments.

Dosage formulation

N. sativa is safe for short-term use in food and medical purposes (Yimer et al., 2019). However, there is insufficient information to determine whether it is safer in higher amount in different health conditions or not. There is no standard dose of *N. sativa* except some different doses studied by researchers. For example, 2 gs (Powder) for 12 weeks has been considering appropriate for most of the respiratory issues. In addition, 500 mg of black cumin oil can also be taken twice daily for 4 weeks (Fallah Huseini et al., 2013). So, it is recommended that people should consult to their healthcare provider before taking *N. sativa* for treatment purpose.

Pharmacological interaction of N. sativa active compounds

N. sativa may interact with concomitant medications and affect intestinal availability and pharmacological effects of co-administered drugs. *In vitro* studies showed that *N. sativa* extract can inhibit or interfere with the metabolism of certain drugs because it has ability to inhibit cDNA expression of substrates mediated by human cytochrome P-450 3A4, 2C9, 3A5 and 3A7. Therefore, *N. sativa* may affect the metabolism of a wide range of drugs (Ali et al., 2012). For example, effect of *N. sativa* on the bioavailability was investigated in rat's upper intestinal sac. In which methanol and hexane extracts of *N. sativa* showed a significant increase in permeability of amoxicillin ($p < 0.001$) as compared with the control at dose depended manner (Ahmad et al., 2013). Further researches are still needed to investigate the pharmacological interaction, and chemical modification in molecular structure of TQ, α-hederin, and other components for the discovery of safer drugs in the future.

Conclusion

The biological compounds of *N. sativa* are widely used as complementary drugs all over the world. Many studies published so far have confirmed pharmacological capabilities of these compounds in regulating inflammatory cytokines during obstructive respiratory disorders e.g. Thymoquinone suppresses mRNA expression which downregulate interferon genes and other inflammatory responses. Similarly, α-hederin suppresses the miRNA-126 expression which consequently interfere with IL-13 secretion pathway. Molecular docking results also showed the superiority of these compounds on FDA approved drugs. These all outcomes unequivocally confirmed the use of *N. sativa* compounds in COVID-19 patients. Further *in vitro* and*in vivo* studies are still needed for knowing their mechanism by which these compounds exert therapeutic effects. Researchers should also investigate the specific and molecular targets of these compounds on cellular level by conducting further preclinical and clinical trials against COVID-19.

Author contributions

All data were generated in-house, and no paper mill was used. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy.

CRediT authorship contribution statement

Muhammad Fakhar-e-Alam Kulyar: Conceptualization, Writing - original draft. **Rongrong Li:** Writing - review & editing. **Khalid Mehmood:** Software, Visualization. **Muhammad Waqas:** Writing - review & editing. **Kun Li:** Formal analysis. **Jiakui Li:** Validation, Supervision.

Declaration of Competing Interest

The authors declare no competing financial interests.

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